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A rapid method for the isolation of genomic DNA from citrated whole blood

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We have developed a DNA isolation method which allows the isolation of high quality DNA from frozen citrated blood and cell lines within 90 min. The procedure avoids the use of toxic organic reagents and tedious extraction steps. DNA prepared by this method is very well suited for restriction fragment length polymorphism and polymerase chain reaction analysis.

INTRODUCTION

The increasing use of genetic methods in clinical laboratories with large series of samples requires methods for rapid and efficient DNA isolation. An efficient DNA isolation method should give high-quality DNA in sufficient amounts, and should be fast and simple. Current methods take 1–2 days, usually including an overnight incubation step with proteinase K followed by phenol and chloroform/butanol extraction [1,2]. Besides being toxic, these organic reagents must be collected and disposed of separately.

Therefore, various modifications of the standard DNA isolation method and new rapid procedures have been published. Many of them cannot be performed from frozen citrated blood without previous isolation of leucocytes, or they include time-consuming extraction steps [3,4]. DNA isolated by some of the rapid procedures is difficult to dissolve, cannot be digested with all restriction enzymes, and is not suited for PCR [5,6].

We have developed a DNA isolation method that allows the preparation of high yields of DNA from frozen citrated blood, buffy coats and cell lines within 90 min avoiding the use of toxic reagents. DNA prepared according to this procedure is excellently cleaved by restriction enzymes and is well suited for PCR.

MATERIALS AND METHODS

Blood samples

Citrated blood was drawn from three individuals, frozen on solid CO_2 and stored at −20 °C until use.

New DNA isolation protocol

Frozen citrated blood (5 ml) is quickly thawed at 37 °C and mixed with 50 ml of cell lysis buffer, pH 8.0 (described by Kunkel [1]). Incubation for 15 min on ice is followed by centrifugation (10 min at 6800 *g* and 4 °C). The supernatant is decanted and the pellet is thoroughly resuspended in 50 ml of washing buffer (10 mM-NaCl/10 mM-EDTA, pH 8.0). After another centrifugation step (10 min at 6300 *g* and 4 °C) the supernatant is carefully siphoned off. The remaining pellet, which should be almost white, is mixed with 350 μl of 20% sodium sarkosyl (*N*-laurylsarcosine) (Sigma), 250 μl of 7.5 M-ammonium acetate, 3.5 ml of 6 M-guanidine hydrochloride (Merck) and 125 μl of proteinase K (10 mg/ml) in the order given. The mixture is incubated at 60 °C until the solution is clear (usually 10–15 min), and then cooled to 0 °C, before the DNA is precipitated by addition of 10 ml of ice-cold ethanol. The DNA is spooled on a hooked glass rod, excess ethanol is gently wiped off and the DNA is dissolved in TE buffer [10 mM-Tris/HCl (pH 7.4)/1 mM-EDTA (pH 8.0)] for 1 h at 37 °C. Generally, under these conditions the DNA is not completely soluble. However, the insoluble material can easily be separated and removed by centrifugation (10–15 s) in a Microfuge B. Good quality DNA is recovered in the supernatant. The DNA can be stored at −20 °C.

Standard DNA isolation protocol

Standard DNA isolation was performed according to Kunkel [1]. Briefly, white blood cells were lysed and treated with proteinase K overnight. Following two phenol extractions and three chloroform/butanol extractions, DNA was precipitated with ethanol.

Controls of the isolated DNA

The isolated DNA was electrophoresed on 0.6% agarose gels in comparison with T_7-bacteriophage (USB) and λ-DNA *Hin*dIII digest (Clontech). Absorbance measurements of the DNA were performed at 260 and 280 nm. The quantity of the DNA was estimated taking one A_{260} unit as equivalent to 50 μg of DNA/ml. The ratio A_{260}/A_{280} served as an indicator of nucleic acid purity.

Restriction enzyme analysis

The isolated DNAs were digested overnight with a series of restriction enzymes (*Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Kpn*I, *Mro*I, *Nar*I, *Pst*I, *Rsa*I, *Sac*I, *Sno*I and *Taq*I from Boehringer Mannheim), using the manufacturer's specifications. The resulting DNA fragments were separated on 0.6% agarose gels in TBE buffer (90 mM-Tris/90 mM-boric acid/3 mM-EDTA, pH 8.0), and transferred to Hybond N membranes (Amersham) by the method of Southern [7]. Prehybridization and hybridization with specific probes was performed according to standard procedures [8].

Probes

For the hybridization experiment an established probe for von Willebrand factor (pVWF 1100, a kind gift from Dr. H. Pannekoeck) [9] was used. The probe was radiolabelled with [^{32}P]dCTP by the random hexanucleotide technique [10]. Autoradiography was performed using Kodak X-ray films S and blue-sensitive intensifying screens.

Polymerase chain reaction

PCR amplification of the DNA region containing the polymorphic *Bcl*I restriction site within the Factor VIII gene was carried out using the primer sequences reported by Kogan *et al.* [11]. The DNA (0.5–1 μg) was isolated as described above. After an initial denaturation for 90 s at 94 °C, 30 cycles, each consisting of 90 s denaturation at 94 °C, 2 min annealing at 45 °C and 2 min extension at 71 °C were carried out. The reaction was concluded with a final extension step for 7 min at 71 °C. Then

30 µl of amplified DNA were digested with *BclI* for 1 h at 50 °C following the manufacturer's specifications. Electrophoresis was performed on a 3% Nu Sieve agarose gel.

RESULTS AND DISCUSSION

DNAs prepared according to our protocol exhibit migration patterns identical to those of samples isolated by the standard phenol extraction procedure. Compared with the markers, T_7 bacteriophage (40 kb) and λ*Hin*dIII digest (23 kb), the size of the DNAs must be at least 20 kb. Fig. 1 shows the agarose gels of the isolated DNAs.

Table 1 gives the yields of the total DNA per 10 ml of whole citrated blood, based on absorbance measurements, and the ratios of A_{260}/A_{280}. The DNA obtained by our protocol is of comparable quality to DNA isolated by the standard procedure, reflected by very similar absorbance ratios.

Using our rapid isolation procedure a significantly higher yield of high purity DNA can be prepared compared to the standard procedure used (sample 1, +140%; sample 2, +167%; sample 3, +76%). The isolated DNAs can be digested to completion with all enzymes tested. As an example, successful digestion by *XbaI* is shown in Fig. 2. The enzyme digests have been blotted and hybridized with pVWF 1100. The autoradiography shows the expected pattern of variable and constant bands (variable bands at 6.9/5.2 kb and 10.5/36.0 kb, and constant bands at 3.8 kb and 2.8 kb). The three individuals are homozygous for the 6.9 kb and for the 10.5 kb bands.

To test their stability, the isolated DNAs have been incubated at 37 °C overnight. An electrophoretic comparison of the incubated samples with the starting material exhibited no qualitative or quantitative difference (results not shown). Our DNA isolation protocol takes only 90 min, which significantly reduces the time required for conventional methods. The procedure is simple to carry out, and avoids tedious extraction steps, toxic reagents and disposal problems of organic solvents.

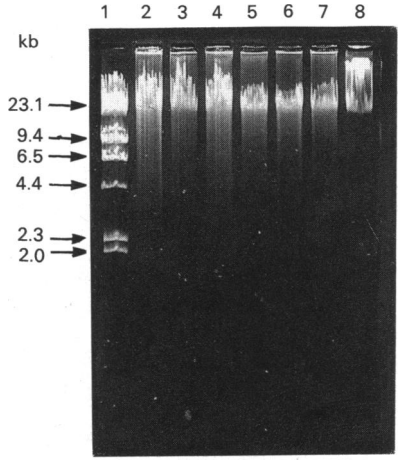

Fig. 1. Electrophoretic analysis of DNA

Abbreviations: std, samples prepared by the phenol-extraction procedure; P, samples prepared by the new ('Parzer') protocol. The isolated DNAs were electrophoresed on 0.6% agarose gels with λ*Hin*dIII digest and T_7 bacteriophage as markers. Lane 1, λ*Hin*d digest (2.5 µg); lane 2, P_3 (1.6 µg); lane 3, std_3 (1.65 µg); lane 4, P_2 (1.5 µg); lane 5, std_2 (1.1 µg); lane 6, P_1 (1.2 µg); lane 7, std_1 (1.3 µg); lane 8, T_7 bacteriophage (2.5 µg).

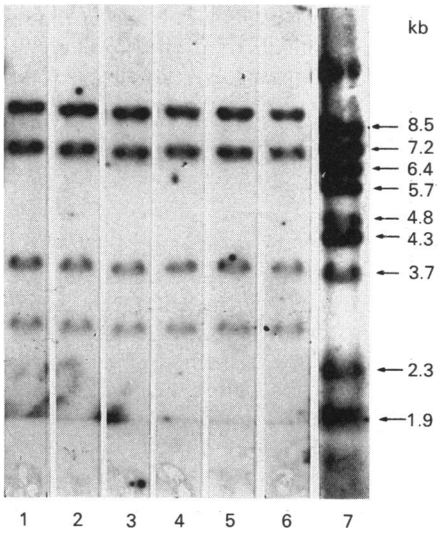

Fig. 2. Southern blot analysis

XbaI digests of the isolated DNAs were run on a 0.6% agarose gel: lane 1, std_1 (5.8 µg); lane 2, P_1 (5.85 µg); lane 3, std_2 (5.1 µg); lane 4, P_2 (5.0 µg); lane 5, std_3 (5.9 µg); lane 6, P_3 (5.83 µg); lane 7, λ DNA *Bst*EII digest (2.2 µg). The separated DNA fragments were transferred to a nylon membrane by Southern blotting. The blots were hybridized with the ^{32}P-labelled probe pvWF 1100. For abbreviations see Fig. 1 legend.

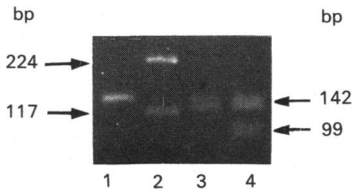

Fig. 3. Polymerase chain reaction

Electrophoresis on 3% Nu Sieve agarose of PCR products after restriction with *BclI*: 10% of amplified unrestricted product was applied to lane 1 and 15% of amplified products were applied to lanes 3 and 4 after restriction. Lane 1, unrestricted PCR product (142 bp); lane 2, λ DNA *Bst*EII digest (2 µg); lane 3, restricted PCR product, subject hemizygous for the absence of the site; lane 4, restricted PCR product, subject heterozygous for the presence of the site.

Table 1. Yields and purities of DNA samples isolated by a standard procedure [1] and a new protocol ('Parzer' protocol)

	1		2		3	
Sample...	Std	P	Std	P	Std	P
DNA (µg/10 ml)	119	286	96	256	217	381
A_{260}/A_{280}	1.55	1.71	1.74	1.68	1.77	1.71

Another major advantage of our method is that the DNA sources (whole citrated blood, buffy coats) can be stored frozen until use. We have already successfully isolated high-quality DNA from buffy coats stored at −70 °C for 4–5 years. In addition, our procedure is also well suited for the preparation of genomic DNA from different cell lines. The isolated DNA can be used for PCR amplification. An example is shown in Fig. 3. The sizes of the amplified products, estimated by reference to λ*Bst*EII size markers, were those expected.

We have proven the versatility of our DNA isolation protocol. Overnight incubation of the DNAs at 37 °C and long-term storage at −20 °C does not cause any alterations concerning molecular mass, concentration or digestibility of the samples. However, we want to point out that blood samples with low leucocyte counts (e.g. from HIV-positive haemophiliacs) have to be treated very carefully. The incubation step with proteinase K has to be watched closely and stopped immediately after the mixture turns clear. Longer incubation results in degradation of the DNA. Therefore we recommend to check this incubation step every 3–4 min.

Generally our method gives significantly higher yields of DNA than the conventional isolation methods. Therefore this DNA isolation procedure has become the routine method in our laboratory and we have applied it with good results during the last 18 months for restriction fragment and PCR analysis.

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